

Annual Project Report 2020

Project title	Developing systems to control male fertility in wheat for hybrid breeding, enhanced pollen production and increased yield		
Project number	21130024		
Start date	01 October 2016	End date	30 April 2021 (requesting extension)

Project aim and objectives

Successful reproduction is critical to grain set in cereals. Thus, optimising and enhancing fertility, alongside controlled fertilisation for breeding and hybrid development, is key to achieve high yields in a sustainable manner. Hybrid vigour increases yield. Therefore, developing hybrid crops offers opportunities to increase productivity. However, this is challenging, especially in wheat due to the need to avoid self-fertilisation. Therefore, mechanisms that control fertility in a reversible manner are needed. There is also a requirement to ensure effective pollination. This relies on high levels of viable pollen for cross-pollination, which is distributed effectively and is resilient to abiotic stress.

This project will address these issues by providing greater understanding of pollen development in cereals by developing switchable systems for the control of wheat fertility, but also by identifying traits for enhanced pollen production and viability, particularly under environmental stress, which are critical for ensuring successful pollination in breeding programmes. We will also determine the environmental stability of fertility control mechanisms in barley and wheat, to enable understanding of the fertility control under different temperatures and light intensities. In addition to providing tools for barley breeding, this work will help in characterisation of the underlying mechanisms for the control of fertility. This is important due to the simpler diploid nature of barley and the greater tools and resources available for barley genetic analysis.

Therefore, by investigating the mechanisms behind these traits and by generating tools for breeding and selection, effective breeding to increase crop productivity and resilience will be realised. The work will also identify the benefit of hybrids for fertility control and the stability of these systems in elite breeding materials.

Key messages emerging from the project

There is conservation of the gene networks involved in pollen development. Genes have been identified from barley and wheat that are critical for pollen development. These are being targeted by mutagenesis to help characterise their function and to generate male sterile lines that could be used for hybrid breeding approaches. In addition, gene editing is being tested for one representative gene, to study the suitability of this technique to produce same phenotype as those generated by conventional mutagenesis. Mutants where all copies of the genes have been mutated are male sterile

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due to failure of pollen development. This phenotype is conserved for the different mutants in barley and wheat lines.

Summary of results from the reporting year

1. Homozygous (Hm) lines for all the homoeologs for all three wheat (Ta) pollen transcription factors (TF) under investigation have been grown and backcrosses are ongoing. Preliminary analyses indicates male sterile phenotypes; one line shows an abnormal growth phenotype that is being investigated. Detailed analysis of these lines is planned, however, materials were lost due to the coronavirus lockdowns and these analyses have been delayed significantly due to the pandemic and access limitations it has imposed.
2. qRT-PCR was developed to determine homoeologs expression pattern for each gene and will be used to study the mutant lines and to compare potential homoeolog expression compensation. We will grow those mutant combinations that are sterile at different conditions to observe whether fertility can be restored. This work has also been delayed due to the coronavirus pandemic and problems of having material for analysis.
3. We have been analysing the impact of environmental conditions on our barley over-expression male sterile line:
 - i) TEM showed increased anther wall thickness and a delay/lack of tapetum layer degeneration within the anther, with irregularly arranged Ubisch bodies. This appears to impact on anther opening.
 - ii) RNAseq analysis has identified some interesting genes essential for anther dehiscence that appears to be downregulated in the sterile overexpression line. Some of these are being targeted for further analysis via CRISPR gene editing.
4. HvTF1: A CRISPR mutant has been identified and the T1 homozygous lines are growing and show complete male sterility. This line is being crossed with the wild type to maintain it and to segregate the Cas9 out.
5. Our manuscript on “Wheat Cadenza floral development staging systems” has been published. (Fernandez et al (2020) J Exp Bot. DOI:10.1093/jxb/eraa156).
6. Our manuscript on “Temperature-dependent sterility based upon overexpression of MS1 in barley” has been published. (Fernandez et al (2020) J Exp Bot. DOI:10.1093/jxb/eraa156).

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7. The HvTF1 over-expression construct has been introgressed into elite barley lines; cross-pollination experiments produced seed; this was assisted due to the floral gapping observed in these sterile lines. This data has formed part of the Temperature-dependent sterility manuscript.

8. Two additional manuscripts on barley transcription factors are in preparation.

Key issues to be addressed in the next year

- Characterise wheat triple homozygous lines in elite backgrounds
- Analyse potential homologue expression compensation in mutant backgrounds.
- Microscopy and functional analysis of mutant lines.

Lead partner	Prof Zoe A Wilson; University of Nottingham
Scientific partners	-
Industry partners	KWS, LIMAGRAIN, RAGT, SECOBRA
Government sponsor	BBSRC

Has your project featured in any of the following in the last year?

Events	Press articles
	Arable Farming magazine: Research in Action Article
Conference presentations, papers or posters	Scientific papers
	Two papers - Fernandez et al (2020) J Exp Bot. DOI:10.1093/jxb/eraa156

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